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14. ABSTRACT

Most(but not all) cases of prion disease are associated with a conformationally altered form of the prion protein (PrP) known as PrPSc. Several lines of evidence indicate that while PrpSc is the infectious molecule, it may not be the proximate cause of toxicity in prion disease. Several other candidates for such a toxic species have been proposed, including an altered topological form of PrP known as CtmPrP. Lines of transgenic mice engineered to express CtmPrP develop a spontaneous prion-like disease. Thus, extending our knowledge of the biology of CtmPrP will likely lead to important clues about how all prion diseases induce neurotoxicity. We have also learned that CtmPrP is much less toxic when expressed on a PrP null genetic background; this result has important implications for the mechanism of toxicity in prion disease. We have used this fact to determine which portions of the PrP molecule interact with CtmPrP to induce toxicity. We have also addressed the role of the Bax protein in CtmPrP-mediated neurological disease. We find that Bax is not required for the disease to develop. We have also begun to examine whether CtmPrP can be studied in a more genetically tractable system, the Baker's yeast Saccharomyces Cerevisiae.

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INTRODUCTION

Prion diseases are commonly associated with the presence of a conformationally altered form of the prion protein (PrP^{Sc}). However, there is mounting evidence that PrP^{Sc} is not directly toxic to neurons; it may require interaction with other gene products to induce a neurotoxic program. One candidate alternate neurotoxic PrP species is ^{Ctm}PrP, which results from an alternate topological decision when the prion protein is translocated into the endoplasmic reticulum. We have identified mutations in the prion protein sequence that dramatically favor the production of ^{Ctm}PrP (which under normal conditions is not detectable). We have established lines of transgenic mice which express ^{Ctm}PrP [designated Tg(L9R-3AV)], and these mice develop a spontaneous neurological illness similar to scrapie [Stewart et al. 2005]. We are characterizing the phenotype of these mice to further our understanding of ^{Ctm}PrP–meditated neurotoxicity, which we believe will shed new light on the process of neurodegeneration in prion disease.

BODY

Task 1: Generation of anti-signal peptide antisera. Months 1-24.

Task complete.

We have proposed creating monoclonal antibodies to the signal peptide, but we have not proceeded with this goal, since previously reported results indicate that ^{Ctm}PrP is not produced at appreciable levels in scrapie-infected animals.

Task 2: Characterization of the ^{Ctm}PrP-induced neurotoxic pathway *in vivo* and *in vitro*. Months 12-36.

We have begun to analyze the molecular mechanism of cell death in the brain by genetic analysis. The Bax protein is recognized as an important regulator of neuronal cell death both *in vitro* and *in vivo*. Previous studies in our laboratory have established a role for Bax function in the cerebellar granule cell death which occurs in transgenic mice expression the PG14 PrP mutation. Surprisingly, while Bax deletion rescued the cell death, it did not rescue the neurological phenotype of these mice [Chiesa et al. 2005]. This result suggests that neuronal dysfunction, rather than cell death *per se*, may be a key feature of prion disease.

We have crossed both Tg(L9R-3AV) C/ PrnP +/+ mice and Tg(L9R-3AV)C/PrnP o/o transgenic mice to Bax knockout (Bax o/o) mice and examined the course of the spontaneous illness. In contrast to the results obtained with Tg(PG14) mice, we see no difference in either the progression of clinical disease or in the severity of cerebellar granule cell loss in terminally ill Tg(L9R-3AV) C/PrnP +/+/ Bax o/o mice compared to Tg(L9R-3AV)C/PrnP +/+/ Bax +/o animals. However, at early stages of the illness, we do observe significantly fewer apoptotic neurons in the Tg(L9R-3AV)C/PrnP

+/+ / Bax o/o mice compared to controls. Our tentative conclusion is that Bax function is not absolutely necessary for ^{Ctm}PrP-mediated granule cell loss, but that it may play some role in the apoptotic process. These results will be finalized for publication in the near future.

We have previously explored the feasibility of a cell-culture based assay to examine the toxicity of ^{Ctm}PrP (as well as other neurotoxic PrP mutants). A robust cell culture assay which will replicate what occurs in the cerebellum of our transgenic mice does not yet exist. However, we are continuing to explore other approaches to make this assay workable.

We have also cultured primary neurons from Tg(L9R-3AV) transgenic mice to attempt to establish a primary cell-culture based assay for CtmPrP toxicity. Unfortunately, the cultured neurons do not show any increased propensity to die in culture compared to non-transgenic controls. Preliminary experiments also suggest that these neurons are not measurably more susceptible to various types of cell stress (such as oxidative stress). Further experiments will be necessary to determine if other factors and/or cell types are required to be present to reproduce the cell death which occurs *in vivo* in a primary cell culture model.

Task 3: Characterization of the cell biology of ^{Ctm}PrP. Months 37-48.

We have studied the cell biology of CtmPrP in cerebellar granule cells in primary cell culture. We find that CtmPrP is localized to the Golgi apparatus of primary neurons, unlike PrPC which primarily localized to the cell membrane. This result is somewhat different from previous studies in transfected fibroblasts, which demonstrated that CtmPrP was localized to the endoplasmic reticulum. We have also directly demonstrated CtmPrP in the Golgi apparatus of intact brain by immunohistochemistry (Stewart and Harris, 2005). These results suggest that the toxic pathway induced by CtmPrP may arise from the Golgi.

Previous studies have shown that one putative function of the normal (PrP^C) form of the prion protein is involved with prevention of Bax-induced cell death [Bounhar et al 2001]. Our laboratory and others have shown that certain mutations in PrP can prevent this Bax rescue function [Roucou et al. 2001; Li and Harris 2005]. We have also established that the phenomenon of Bax rescue by mammalian PrP can be replicated in the baker's yeast *Saccharomyces cerevisiae*. These results allow the mechanism of the rescue to be examined in a genetically tractable organism.

We have introduced PrP mutants which favor ^{Ctm}PrP formation in mammalian cells into yeast and asked whether they also function in the Bax rescue assay. Some of the mutants do rescue efficiently, but some do not. Specifically, mutations in the N-terminal signal peptide of PrP abolished Bax rescue. Confirmation that the PrP topology decisions occur in yeast as they do in mammalian cells will follow in the near future. If these results are confirmed and correlate with the presence of ^{Ctm}PrP, a further structure-function study of PrP in yeast will be performed to determine which portions of the PrP molecule are directly responsible for the Bax rescue activity. Future work will also determine which intracellular compartments ^{Ctm}PrP occupies. Previous Progress reports have described ^{Ctm}PrP residence in the Golgi apparatus in primary cultured neurons and certain cell lines [Stewart and Harris, 2005]. It will be of interest to determine if this cellular localization is conserved in yeast.

Task 4: Structure-function analysis of ^{Ctm}PrP using chimeric proteins. Months 42-60.

Not begun.

However, based on the preliminary data from yeast, we plan to examine some of the chimeras proposed in Aim 4, initially in yeast, then in mammalian cells. Possibly some of the more informative chimeras may be used to create transgenic mice to assay their effects in vivo.

We have also begun a different but related structure-function assay using presently available transgenic mice. These experiments are intended to answer a slightly different question: Which structural/sequence elements of endogenous PrP are required to interact with ^{Ctm}PrP to induce spontaneous neurological illness? We have previously shown (as described in previous Progress reports) that the illness induced by ^{Ctm}PrP expression is at least partially dependent on endogenous PrP expression [Stewart et al. 2005]. When the Tg(L9R-3AV)B mouse line was bred onto a PrnP o/o genetic background, the spontaneous illness no longer developed. We have crossed these Tg(L9R-3AV)B/ PrnP o/o mice to two different mouse lines expressing altered PrP transgenes to determine if these altered PrPs are sufficient to restore illness.

The first mutant line is designated Tg(D11) [Schmerling et al 1998]; it expresses a PrP deleted from amino acids 32-80; thus it is missing most of the octapeptide repeat region of PrP. These Tg(L9R-3AV) B/Tg(D11)/ PrnP o/o mice do not show any spontaneous illness at greater than 365 days. Thus, we tentatively conclude that the octapeptide region of PrP is essential for the ^{Ctm}PrP–PrP^{C} interaction to occur.

A second mouse line has recently become available. Tg(GPIneg) mice express PrP , but abolish the GPI anchor addition site in PrP. Thus, they secrete PrP readily into the extracellular environment [Chesboro et al. 2005]. We have crossed Tg(L9R-3AV) B/PrnP o/o mice to these Tg(GPIneg) mice to determine if the WT PrP function requires PrP to be present on the cell surface of neurons in a membrane-anchored form. These results are in progress. Additionally, we will cross the Tg(L9R-3AV) B mice to mice expressing the wild-type prion protein (designated E1 mice) as a positive control for the introduction of PrP by a transgenic vector.

KEY RESEARCH ACCOMPLISHMENTS

-Breeding of Tg(L9R-3AV) mice to Bax null mice.
-Breeding of Tg(L9R-3AV) mice to Tg(D11) and Tg(GPIneg) mice.
-Expression of ^{Ctm}PrP-inducing PrP mutants in yeast.

REPORTABLE OUTCOMES

-Breeding of Tg(L9R-3AV) mice to Bax null mice- manuscript in preparation.

CONCLUSIONS

We have established a mouse model for spontaneous illness induced by an alternate topological isoform of the prion protein (PrP). We have employed these mice to further explore the cellular mechanisms of neuronal pathology induced by ^{Ctm}PrP. We believe these mechanisms will yield new information on the neurotoxic mechanisms in prion disease, a subject about which relatively little is known. With this information better therapeutic treatments may someday be developed.

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